Cardiac Myosin Binding Protein-C in Sprague Dawley Rats exposed to Sub-Chronic oral dose of Cadmium Chloride

Enjoud Khalifa Al-Borshaid, Kavitha Varadharajan, Muralitharan Shanmugakonar, Hamda Al –Naemi
Laboratory Animal Research Centre (LARC), Qatar University, Doha, Qatar

Faculty and PostDoc
Health and Biomedical Science

Introduction

Cardiac myosin binding protein C (cMyBP-C) plays a vital role in contractility of the cardiac muscle. Under pathological conditions such as cardiac infarction, dephosphorylation of the protein implicated in the decrease of cMyBP-C levels. Cadmium toxicity has been associated with heart diseases linking it to the level of cMyBP-C in the heart. Due to high mortality rate from cardiovascular diseases and elevated levels of industrial pollution in Qatar, it is of importance to study such a link between these two factors exists. This study was carried out to investigate the link between cadmium toxicity and the levels of cMyBP-C in the hearts of laboratory rats and the impact on the occurrence of cardiovascular disease. Heart samples from two groups of SD rats (control; n=6 and treated; n=6) were subjected to both protein and gene expression analysis. Treated samples showed non-significant down regulation of cMyBP-C gene and protein expression in comparison to control group.

Tissue Processing:

- Tissue samples of 10 weeks of cadmium chloride exposure collected from LARC sample archive and processed for the study.
- Heart tissue chopped finely after weighing and transferred to 1.5 mL microfuge tube for RNA and protein extraction individually.
- Respective Trizol & RIPA buffer added and sonicated using cell sonicator.

Protein Extraction & Western blot Analysis:

- Total protein extracted by RIPA cell lysis method and quantified by Bradford assay.
- Total protein separated by SDS-PAGE analysis.
- Separated protein were transferred using PVDF membrane & probed with cMYBP-C primary & secondary antibodies that detected by ECL system

RNA Extraction & Gene Expression Analysis:

- Total RNA extracted by trizol method and quantified by Nanaophotometer.
- A known amount of RNA was converted in to cDNA for gene expression analysis using Taqman reagents and specific primer by Quantstudio 6 flex qPCR machine.

Protein Separation by SDS-PAGE Analysis

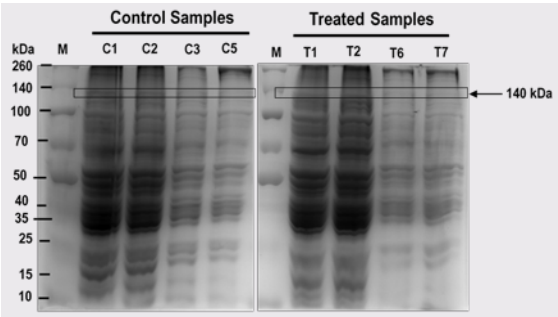

Figure 1. illustrates the SDS- Polyacrylamide gel electrophoresis of total protein extracted from heart tissue samples confirms the presence of protein in the targeted molecular weight (140kDa).

Protein Expression Analysis

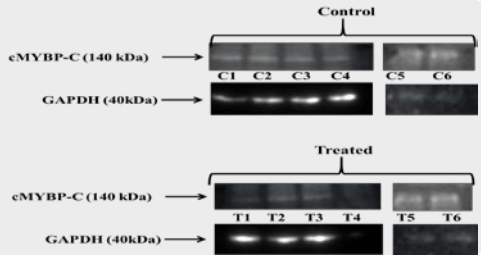

Figure 2. Western blot analysis of whole cMyBP-C (140kDa) compared and the endogenous control GAPDH (40kDa) compared against control samples

Gene Expression Analysis

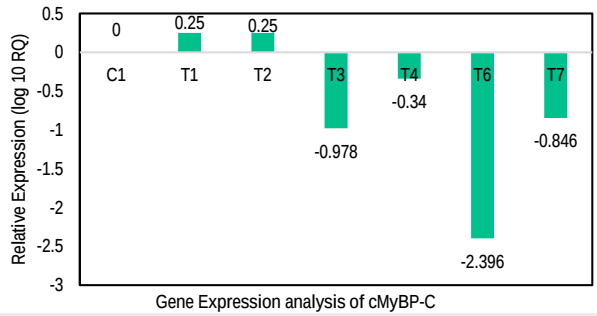

Figure 3. describes the gene expression analysis of cMYBP-C.

The relative quantification (RQ) of gene is considered significant if there is at least two-fold change when compared to control

Samples T1 and T2 shows upregulation and samples T3, T4, and T7 show down regulation, which has non-significant alteration on the gene, but sample T6 shows significant down regulation

Conclusion: Cadmium cardiotoxicity has an impact on both protein and gene expression. Changes observed in both gene and protein analysis however the changes are not at significant level. This study adds the knowledge and showed for the first time the effect of Cd as a toxic heavy metal on the functional state of cMyBP-C. Further studies need to be done to understand the mechanism by which Cd causes the breakdown of cMyBP-C, and how the protein could be conserved. This leads to an understanding and development of therapeutic strategies to treat the damage of cardiac muscle caused by Cd.

Acknowledgments: This research is supported by fund granted from Qatar National Research Fund (QNRF) Grant No: UREP24-175- 3-051.